Genome Editing
Opportunities for reduction?

Sara Wells

MRC Harwell Institute- Mouse genetics

Molecular and Cellular Biology

Generation of new GA lines and archiving

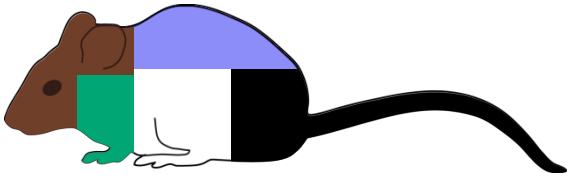
Breeding and Colony Management

Phenotyping and *in vivo* experiments

Ex-vivo testing and Pathology

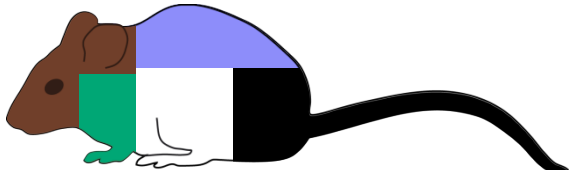
Genome editing: Supporting the generation of mouse models for biomedical research

- Knock out alleles
- Mutagenesis at scale
- Complex alleles



Genome editing: Capacity, Allele validation and versatility

- Deletions/Point mutation/Base insertion
- Mouse models with mutation thought to be causative of human disease, GWAS candidates, …
- Integration of small cassettes (loxP, tag) in embryos
- Large locus integration (i.e. Humanisation)
- ES cell and one-cell embryo
- Any genetic background
- Increasingly versatile and efficient

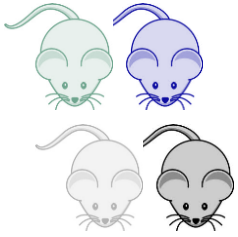


CRISPR-induced mutants: an assembly line

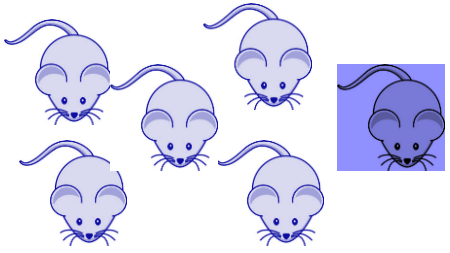
Design of mutation and guides

Cloning and transcription of reagents

Micro-injection



Screening of F0s (mosaic)



Sequencing of F1s

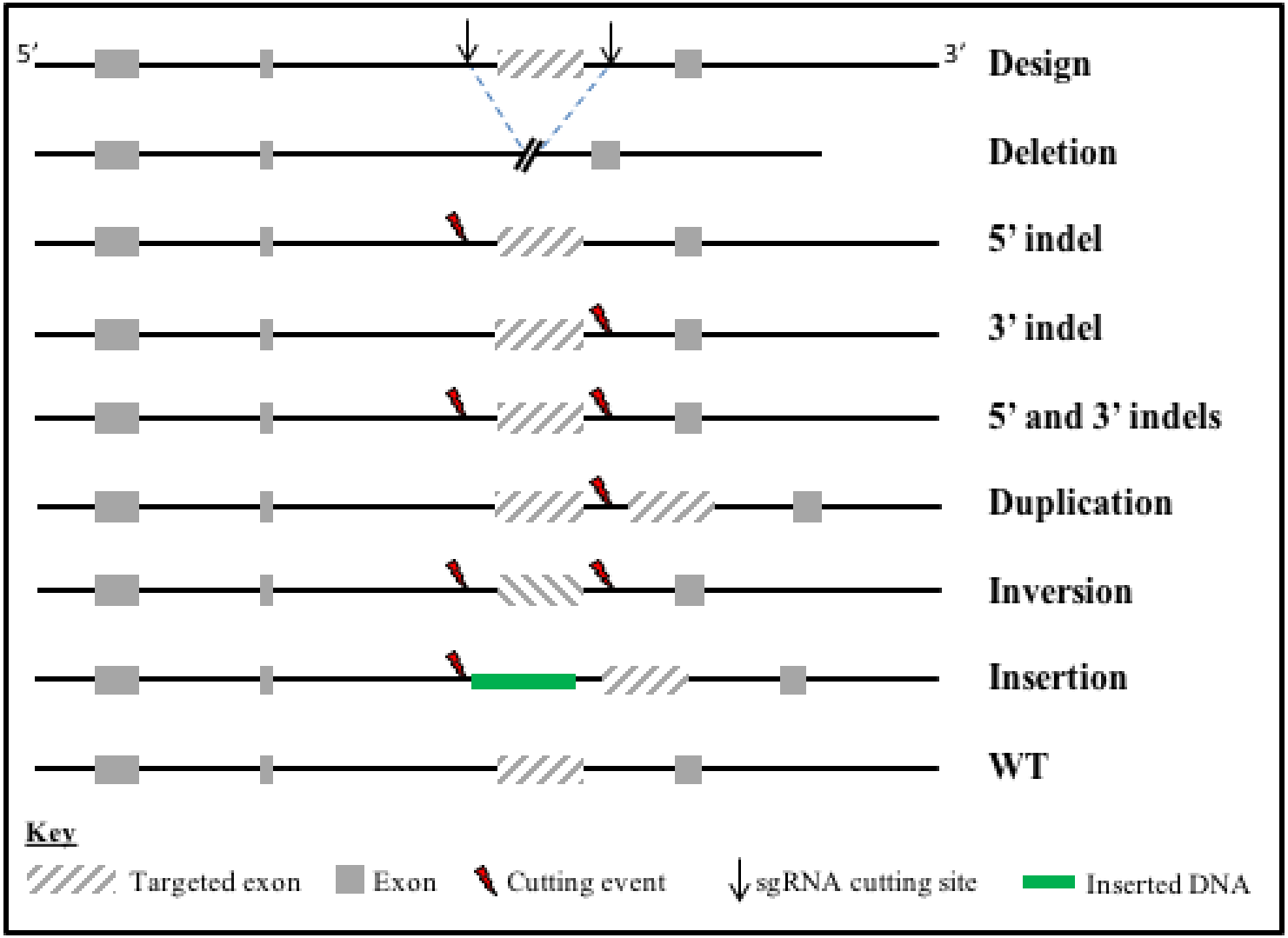
4 months

Genome Editing capacity: 150 new lines/year

- Over 250 projects in the pipeline, 180 analysed:

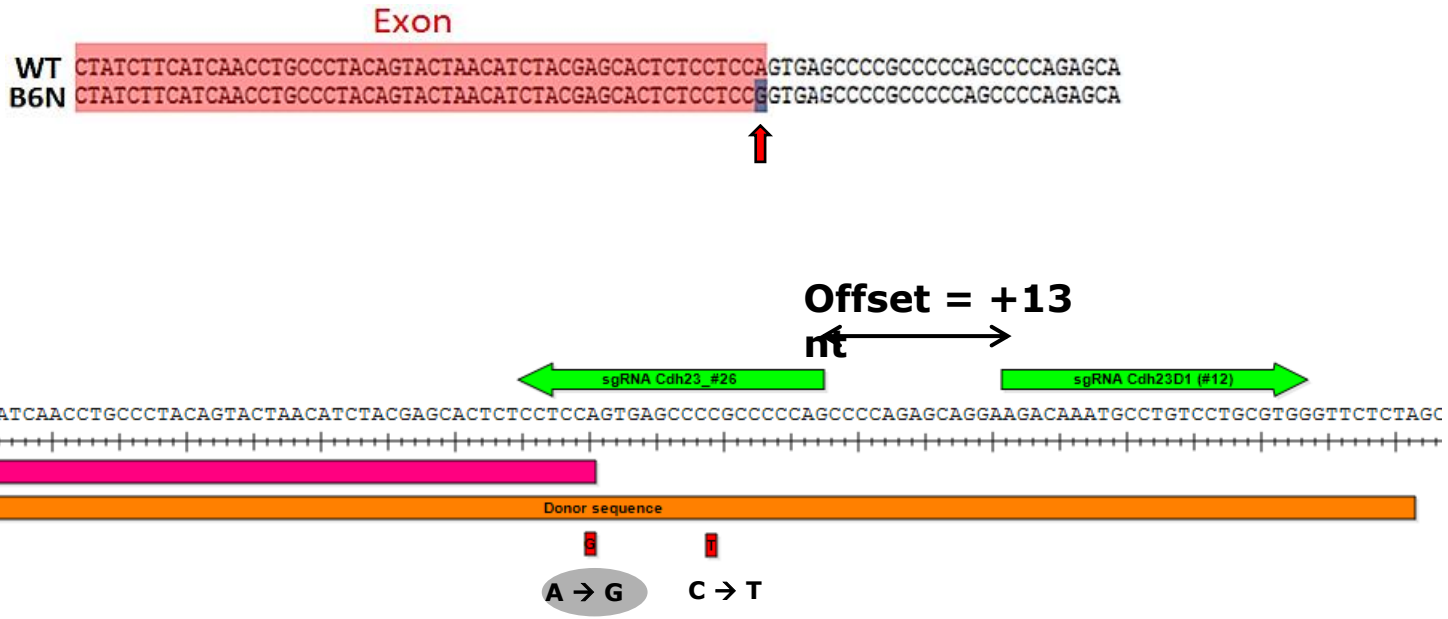
	Indel	Deletion	PM and other HR
Percentage of mutated founders / F0 born	**38.5**	**ND**	**24.6**
Percentage of expected mutants / F0 born	**28.5**	**15.7**	**6.8**
Frequency of animal with desired mutation	**1/3.5**	**1/6**	**1/15**

Deletions with CRISPR can yield many artefacts



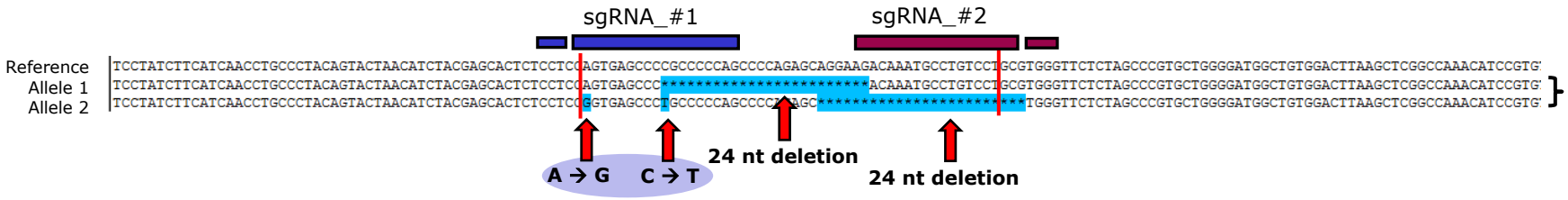
Generation of a point mutation with CRISPR

- Mutation *Cdh23*^{753A} = synonymous SNP in exon 7 which causes in-frame skipping of exon 7
  - ✓ SNP associated with Age-related Hearing Loss (AHL)



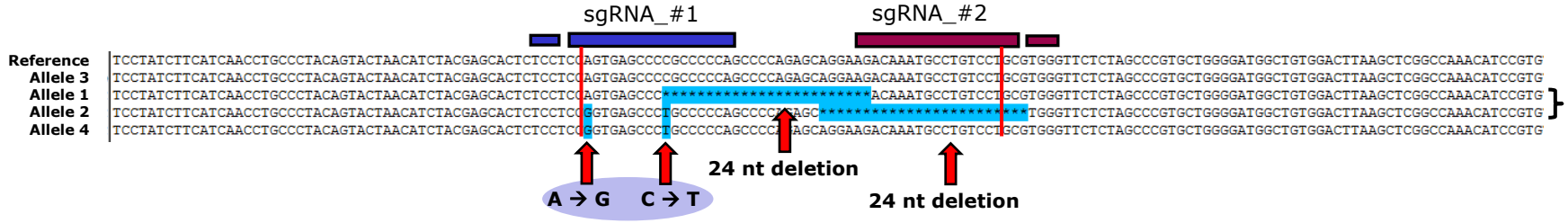
Mosaicism in F0 mice and illegitimate repair

Cdh23 target, F0 #30 ear clip genotyping results:



- Allele 1 = NHEJ repair → 24 nt deletion
- Allele 2 = Illegitimate repair → Correct repair at the target + 24 nt deletion

Cdh23 target, alleles found in F1s (#30 offspring):



- Allele 1 = NHEJ repair → 24 nt deletion
- Allele 2 = Illegitimate repair → Correct repair at the target + 24 nt deletion
- Allele 3 = WT
- Allele 4 = HDR → Correctly repaired

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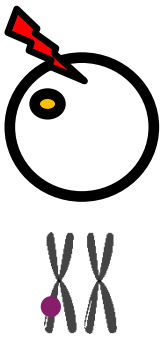
Genotype complexity and mosaicism

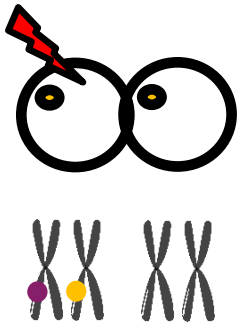
- Cas9 mRNA
- sgRNA(s)
- DNA donor template

Pronuclear injection in 1 cell stage embryos

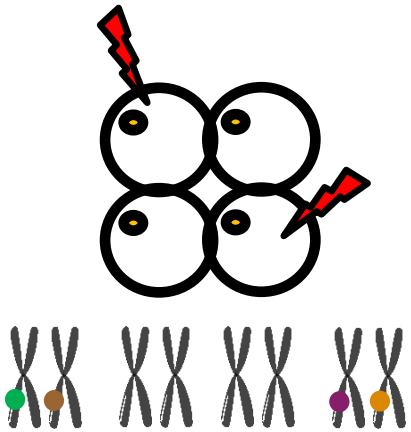
= Mutagenesis event



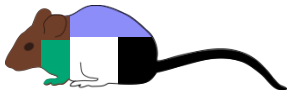
Mutant heterozygous
(2 alleles)



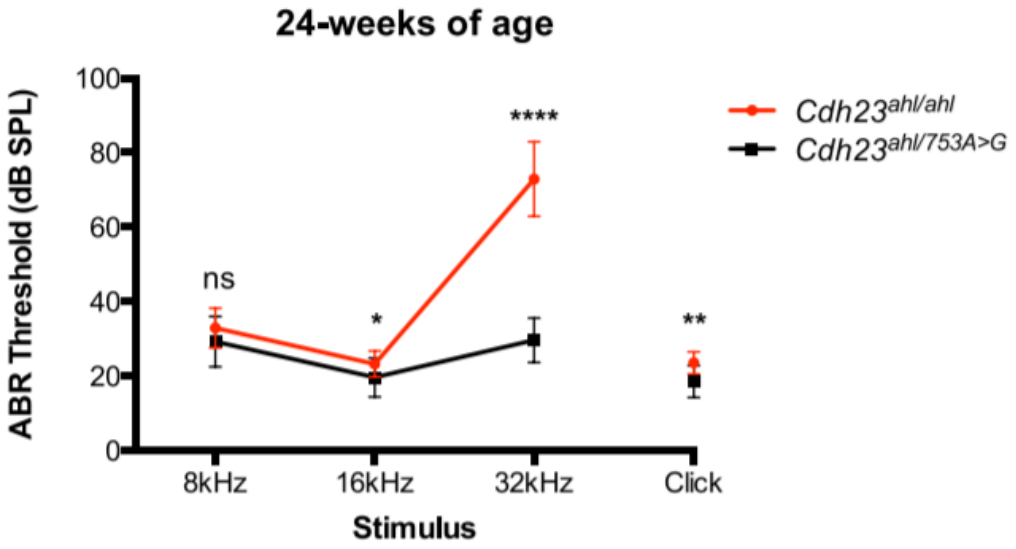
Mutant mosaic
(3 alleles)

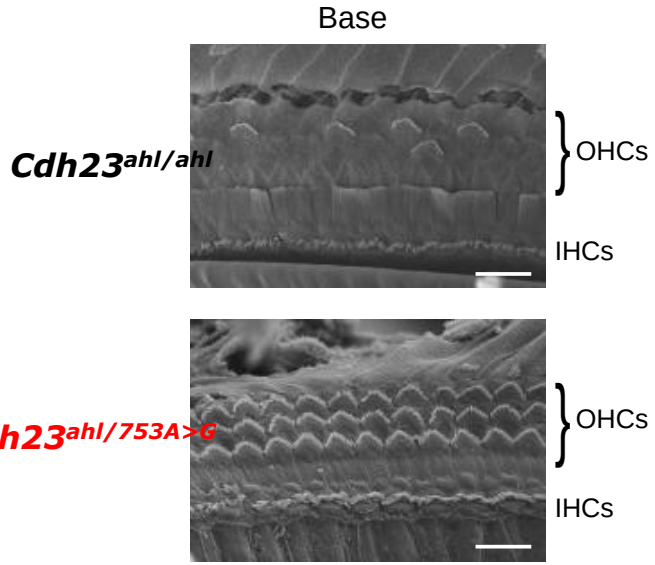


Mutant mosaic
(5 alleles)



Correcting inbred defects

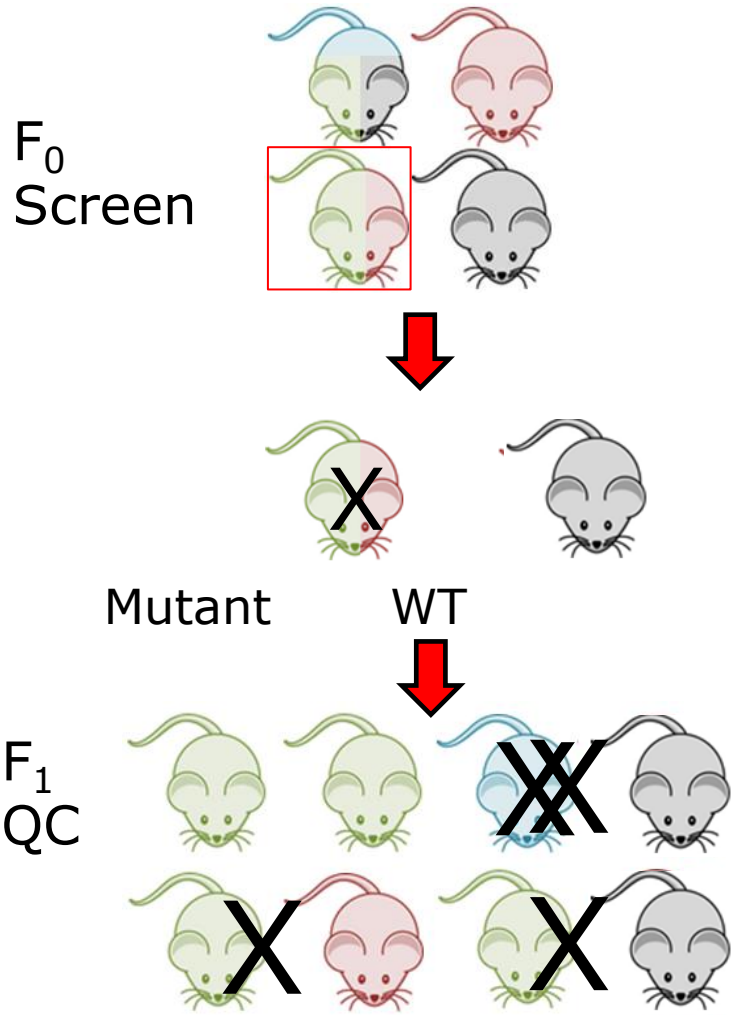




Cdh23$^{ahl/ahl}$ is the genotype of the background strain and is not regulated.

Cdh23$^{ahl/755A>G}$ is currently regulated, although we can apply to have it removed from the act.

Genome Editing screening and validation



Small indel, Point Mutation

- Sequencing of all F_0s
- PCR products sub-cloning and sequencing for selected animals

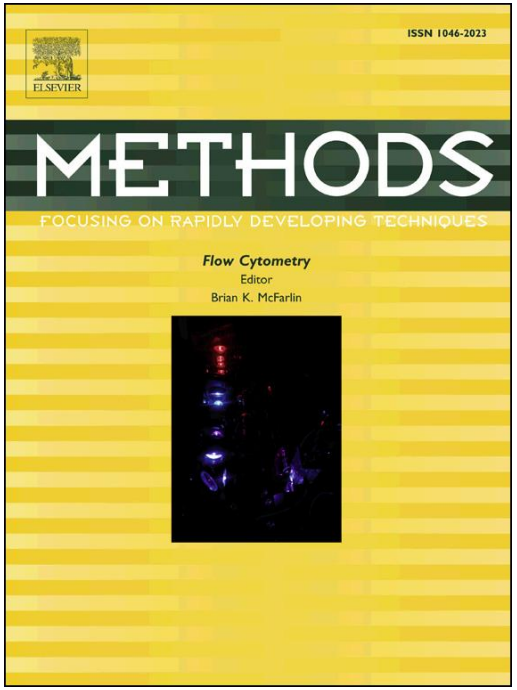
Deletion

- PCR across deleted fragment

All allele types

- Whenever possible, PCR-based pre-screen
- Sequencing of all F_1s to be carried forward

Screening of F_0s - Genotyping of F_1s



Analysing the outcome of CRISPR-aided genome editing in embryos: Screening, genotyping and quality control

Joffrey Mianné, Gemma Codner, Adam Caulder, Rachel Fell, Marie Hutchison, Ruairidh King, Michelle E. Stewart, Sara Wells, Lydia Teboul

PII: S1046-2023(16)30270-5
DOI: http://dx.doi.org/10.1016/j.ymeth.2017.03.016
Reference: YMETH 4168

To appear in: *Methods*

Phenotyping of F_0s?

Mamm Genome (2017) 28:377–382
DOI 10.1007/s00335-017-9711-x

CrossMark

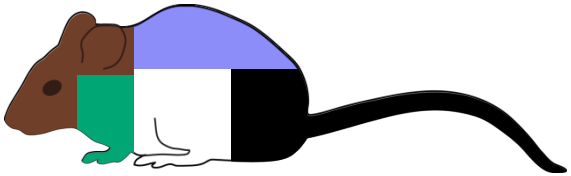
Phenotyping first-generation genome editing mutants: a new standard?

Lydia Teboul[1] · Stephen A. Murray[2] · Patrick M. Nolan[3]

Received: 22 June 2017 / Accepted: 14 July 2017 / Published online: 29 July 2017

CRISPR/cas9 aided mutagenesis: Lessons learned

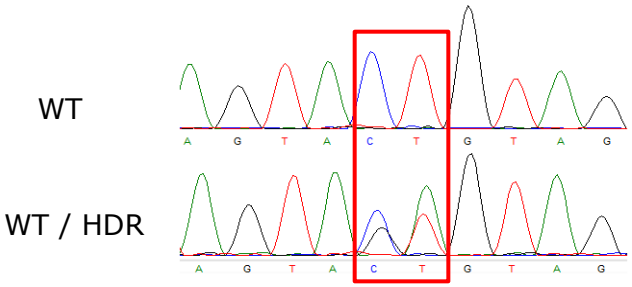
- Repairs following CRISPR cut are error-prone: “illegitimate repairs”, rearrangements. Quality control of the alleles obtained is essential and complex.
- F0s should be treated as mosaic.
- Phenotype data should be acquired from F1 onwards, once the alleles have been segregated and fully characterised (screens can be an exception).



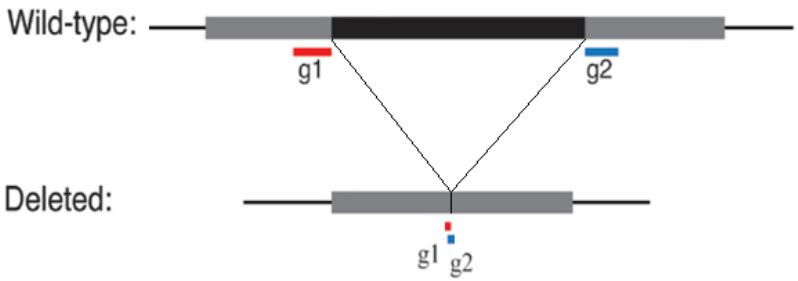
Extending genome editing capacity

- **Indel(s) / Knock-out(s)** ✔

- **Point mutation(s)** ✔

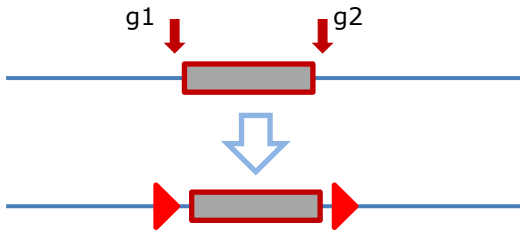


- **Tailored deletion(s)** ✔



- **Gene targeting** . . .

Ex: Flox



Genome editing: Fast evolving technology

SCR7

Rad51

RS-1

Embryo electroporation

Cas9 endogenous expressor

Other Cas9

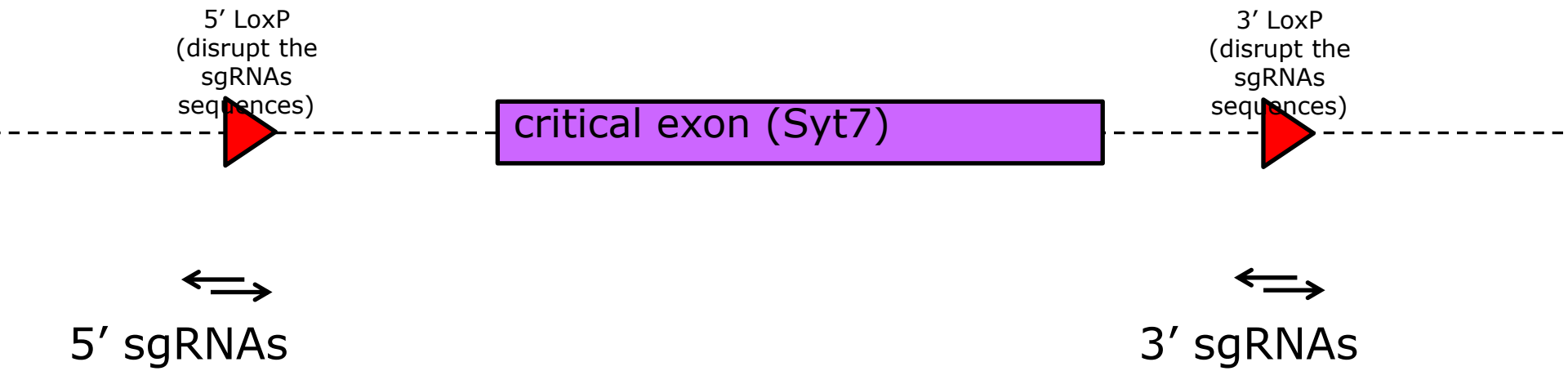
Chemically modified oligos

Protein *versus* mRNA

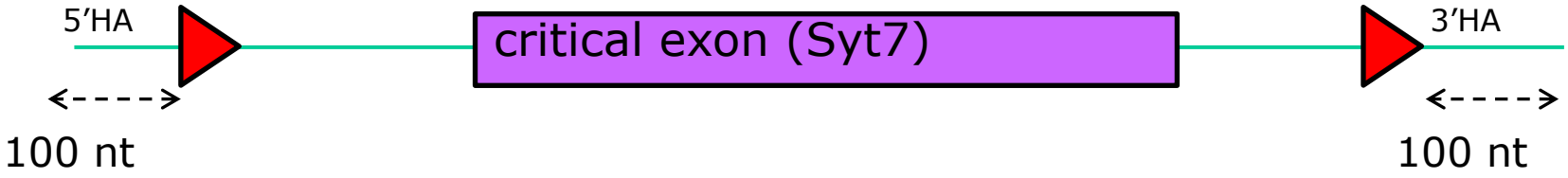
Long ssDNA donor

MR

Long single stranded DNA donors
Conditional design



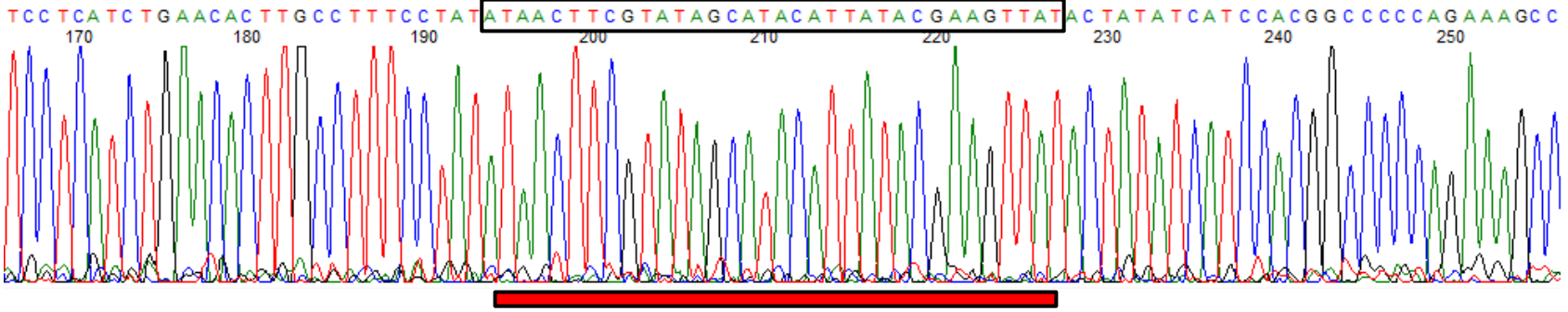
Donor: long ssDNA:



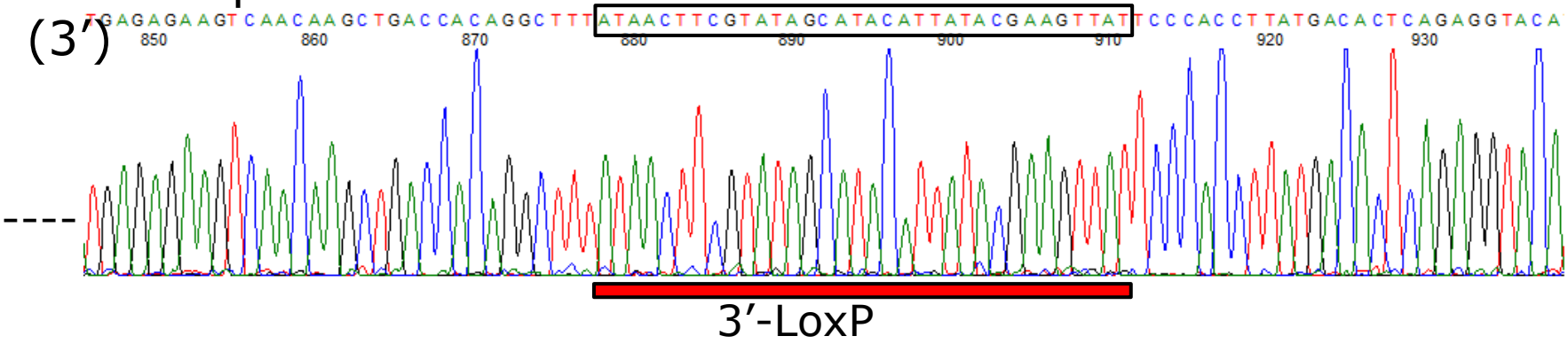
Conditional Tm1c first results: Syt7

- F_0 ♯2 → Homozygous for the repair?

Forward primer (5’)



Reverse primer (3’)

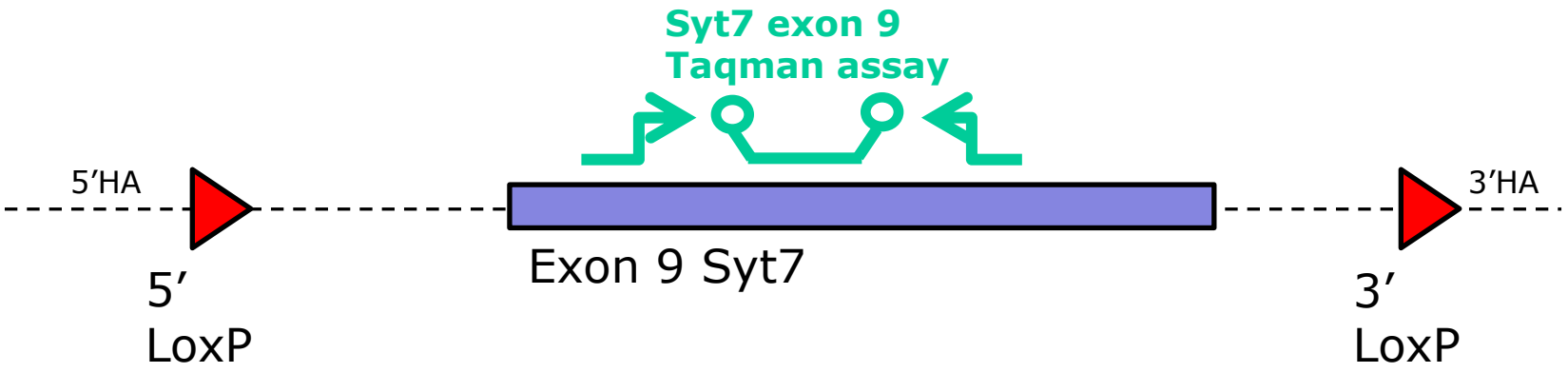


Critical region all

ddPCR screening for random insertion

- LoxP PCR informs of the presence/absence of 3’ and 5’ LoxP sites, but unable to indicate whether there are random integrations/large deletions present.

- TaqMan assay centred on CR through ddPCR



Analysis of Syt7 cKO – line 2

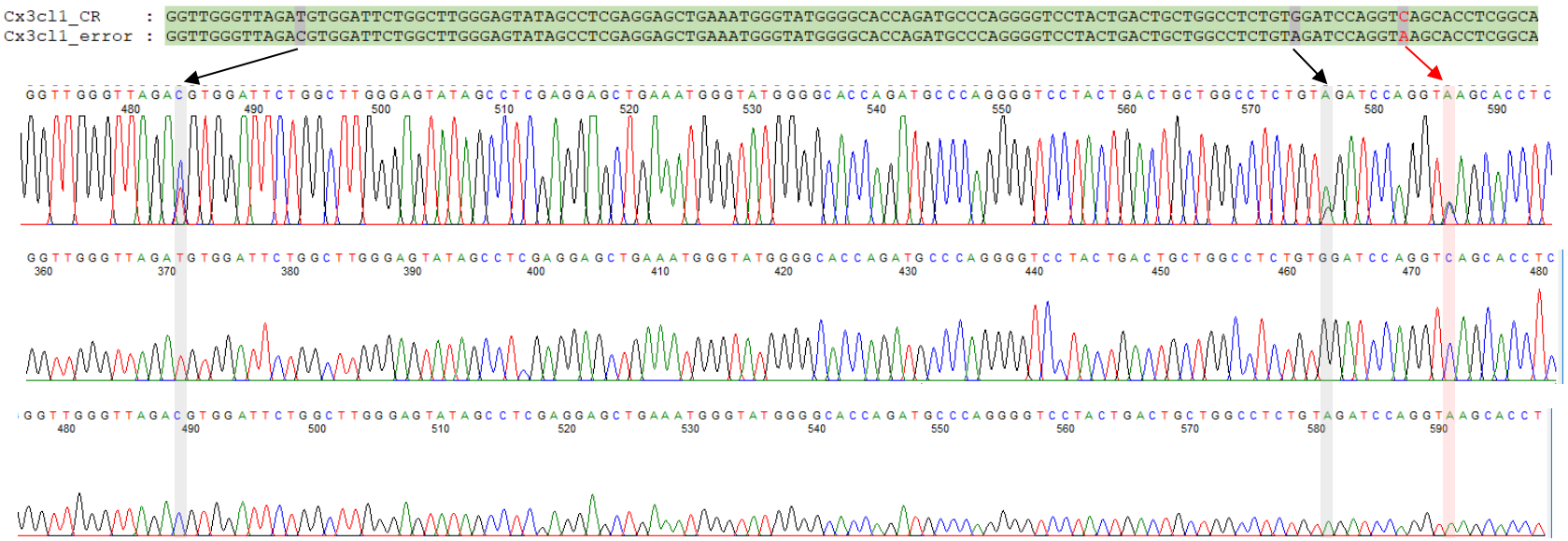
Animal	PCR Result	ddPCR result
F_0 ♯2	Correct mutant: Homozygous	2.78 (3 copies)

Breeding gave rise to 8 F1 mice

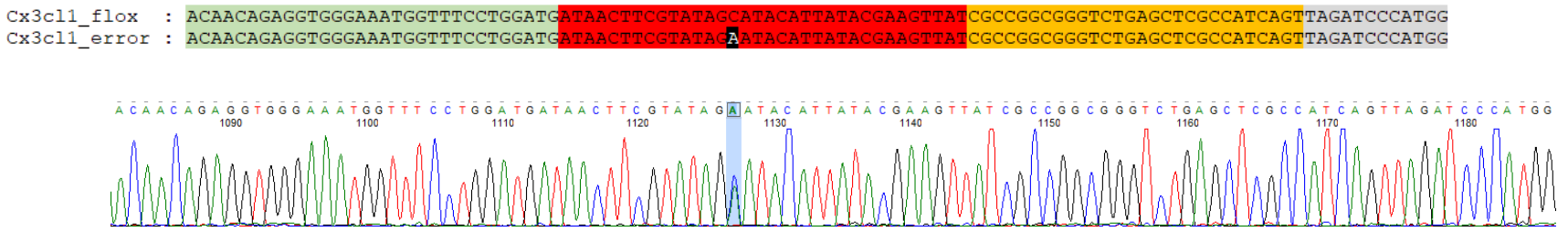
Animal	PCR Result	ddPCR result
♯2 offspring 1	WT	1
♯2 offspring 2	WT	2
♯2 offspring 3	Correct mutant	3
♯2 offspring 4	WT	3
♯2 offspring 5	Correct mutant	2
♯2 offspring 6	Correct mutant	2
♯2 offspring 7	Correct mutant	3
♯2 offspring 8	WT	3

Additional base changes

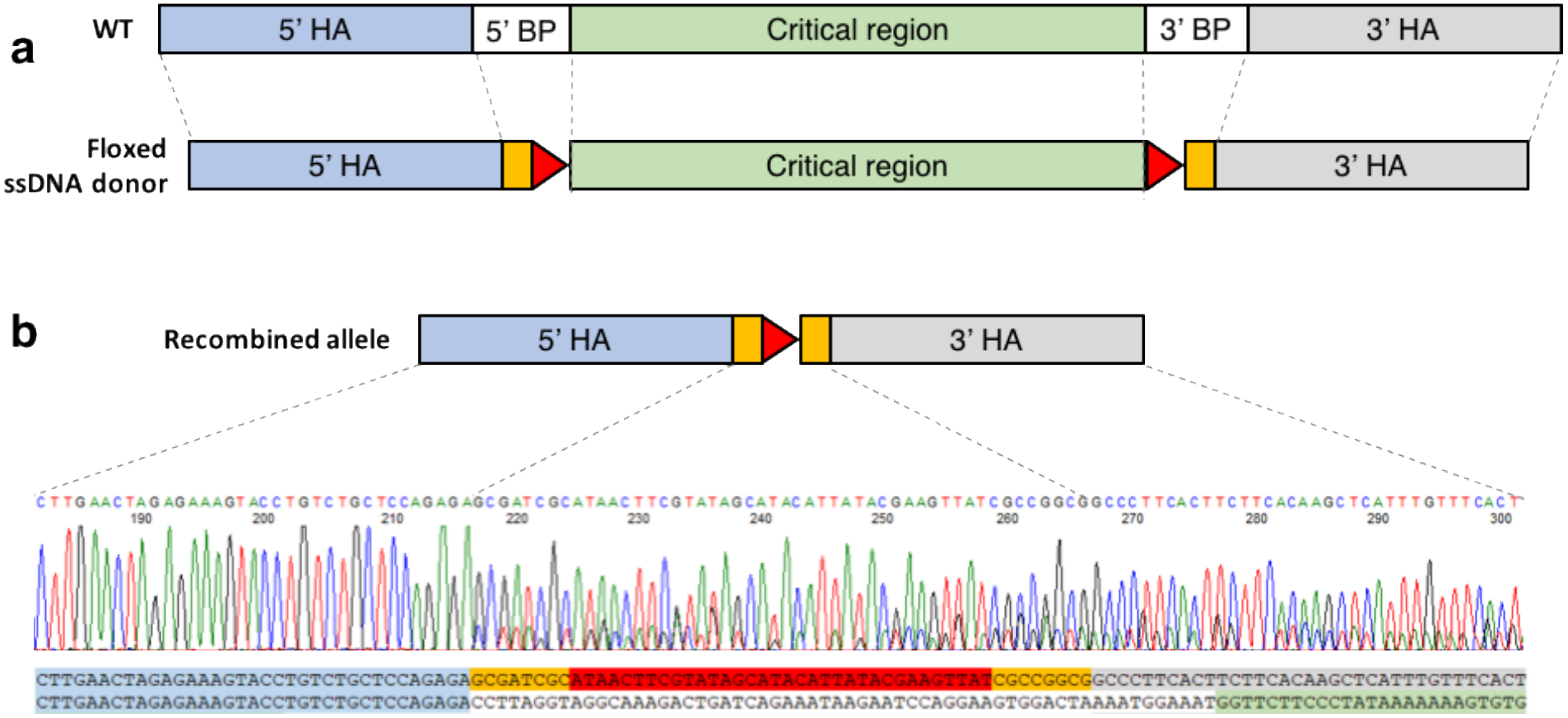
b



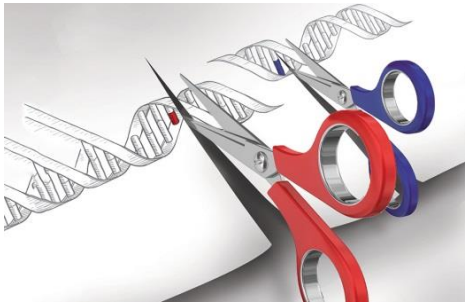
c



Allele rearrangements



New opportunities for mouse models



Genome Editing
- Humanised genes
- Modified existing models
- Modified backgrounds
- Controlled heterogeneity

Breeding and Archiving
- Large cohort breeds
- Oligogenic breeds
- Archiving of intermediate generations

Phenotyping
- Automated platforms
- Data capture LIMS
- Analysis pipelines

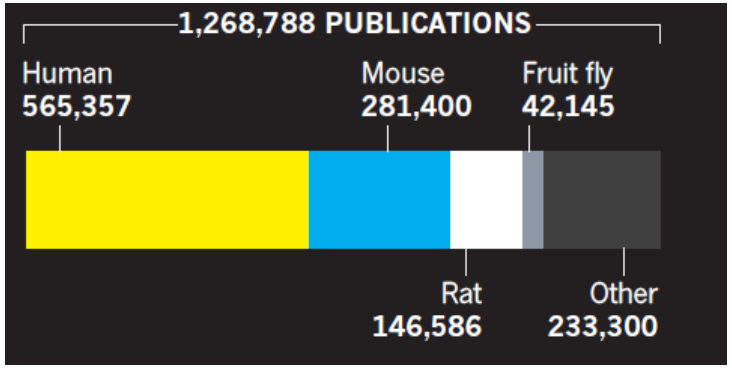
THE GREATEST HITS OF THE HUMAN GENOME

A tour through the most studied genes in biology reveals some surprises.

THE TOP 10

The ten most studied genes of all time are described in more than 40,000 papers.

1	*TP53*	8,479 citations
2	*TNF*	5,314
3	*EGFR*	4,583
4	*VEGFA*	4,059
5	*APOE*	3,977
6	*IL6*	3,930
7	*TGFB1*	3,715
8	*MTHFR*	3,256
9	*ESR1*	2,864
10	*AKT1*	2,791



Out of the 20,000 or so protein-coding genes in the human genome, just 100 account for more than one-quarter of the papers tagged by the NLM. Thousands go unstudied in any given year.

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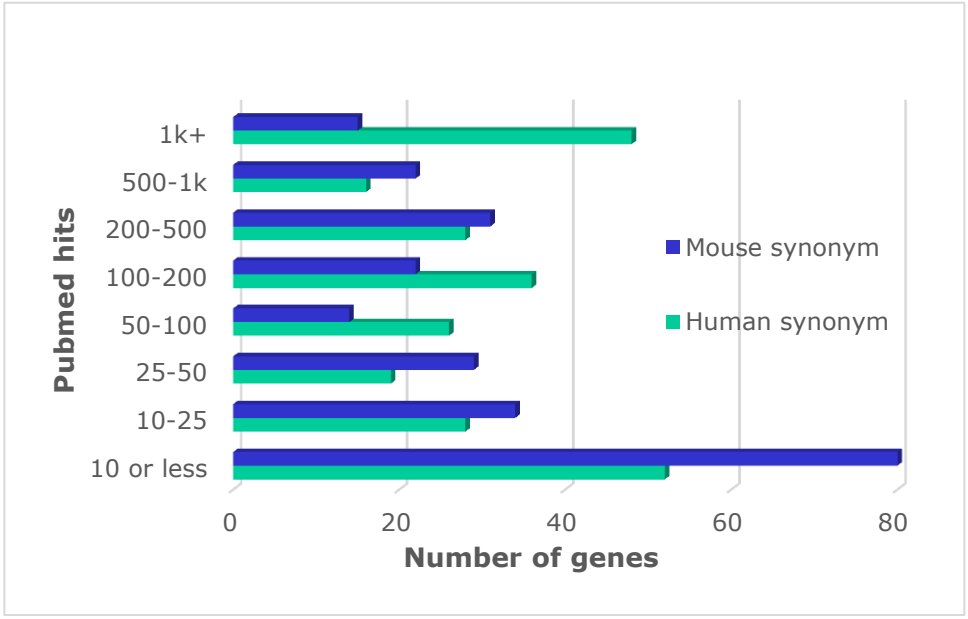
Mice and many many genes

- There are mouse models for less than 50% of genes
- There are many different models foe lots of the same genes
- There are many papers on selected models of mice
- (Apo E KO mice= over 6k papers)

This is just the start…

There's the rub: it takes a certain confluence of biology, societal pressure, business opportunity and medical need for any gene to become more studied than any other. But once it has made it to the upper echelons, there's a "level of conservatism with certain genes emerging as safe bets and then persisting until conditions change".



Distribution of IMPC stocks from Harwell

Wider use of mouse models

FOUNDATION FOR PRADER-WILLI RESEARCH

Working Toward an Independent Future

Genomics england

UK Dementia Research Institute

Modelling Human Mutations

Genomics england

100k genomes
Cancer, infection and rare diseases

+ support data

Preclinical studies

sequence

mouse

Diagnosis

An International Centre for Mouse Genetics

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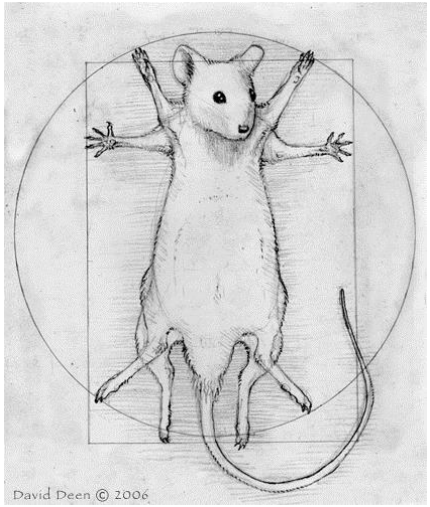
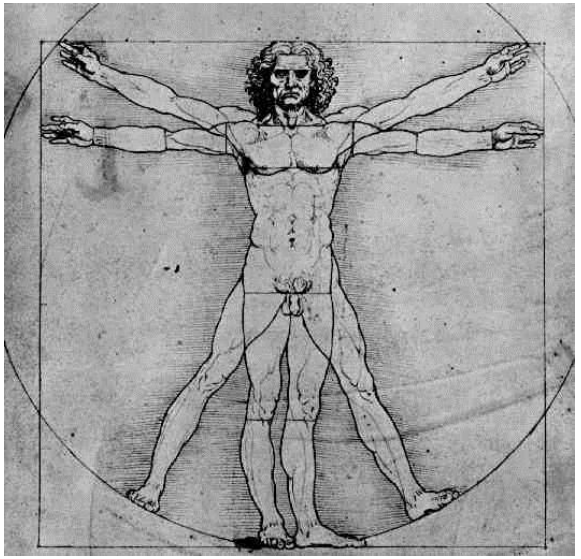
Humanising the mouse

Genes, Physiology and Behaviour = same but not identical!!!

- Mice aren't little humans
- The differences are important



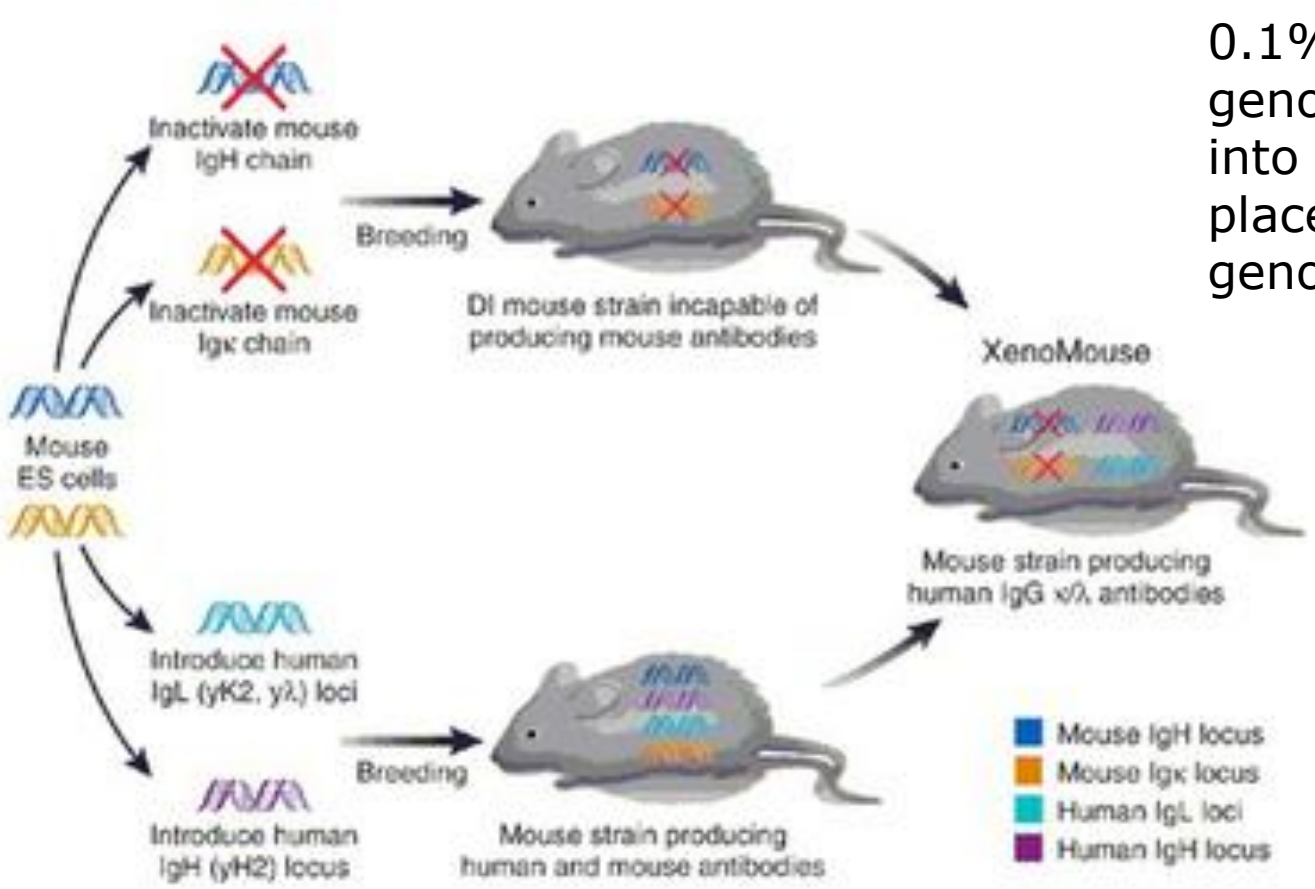


Can we close the gap?

Critique of the mouse as a model system

- **Knockouts don’t model human gene changes**
- **Mouse data doesn’t translate to human drug trials**

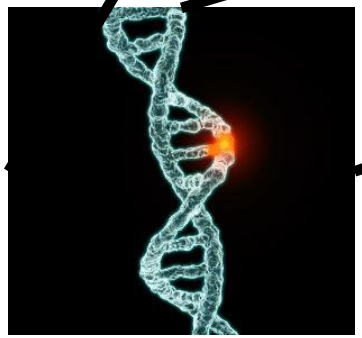
Humanising mouse genes

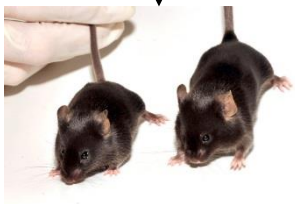


0.1% of the human genome inserted into the appropriate place in the mouse genome

Kymouse™ has full human antibody system

Controlled Heterogeneity

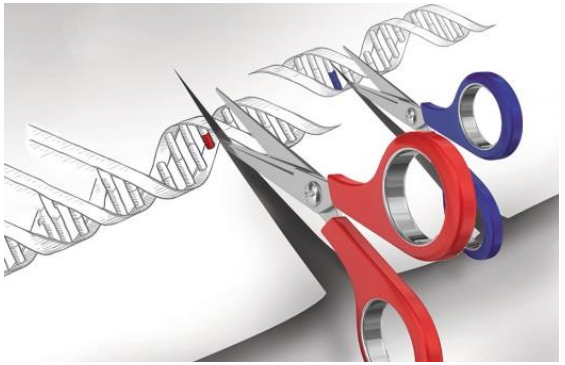




The New Age of Genetically Altered (GA) mice (another one!)

Genome Editing (CRISPR/Cas9)

- Rapid establishment of genome editing
  Expansion of Molecular Biology teams
- Change in the type of models generated
  - More diverse
  - More refined
  - Potentially more unpredictable



What differences will we see in animal facilities??

- More complex crosses
- More different lines
- More unpredictability

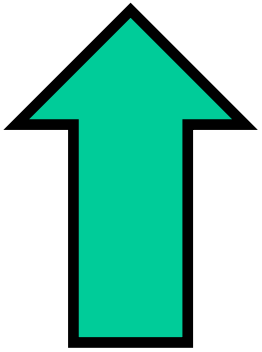
Mousing human behaviour??

Should we be doing certain tests at night???

- **Anxiety**
- **Cognition**

Summary

- Animal numbers likely to increase as a result of genome editing
- More species



- Sophistication of mouse alleles
- Refinement towards human models
- Better translatable science that in the long term- will reduce animal numbers